Hidehisa KOBA*: A contribution to the taxonomy of *Poa nipponica* and related species (Poaceae)

木場英久*: オオイチゴツナギとその近縁種（イネ科）の分類学への寄与

Poa nipponica and related species are small to medium sized annual or short-lived perennial grasses. They are ubiquitous around human habitations in Japan except for Hokkaido. Species in this group commonly possess hairy keels in palea, and belong to the Section Ochlopoa.

Ohwi (1941) recognized the following five species in this species aggregate in Japan: *Poa nipponica* Koidz., *P. acroleuca* Steud., *P. hisauchii* Honda, *P. annua* L., *P. crassinervis* Honda. This treatment has generally been adopted since then (e.g. Koyama 1964, Tateoka 1985). Of the five species, *P. annua* has world-wide distribution, *P. crassinervis* is endemic to south-western Japan (Tateoka 1987), and the remaining three species are eastern Asiatic in range (Ohwi 1941, 1975). Ordinary forms of these five species each have distinctive morphological features, and it is not difficult to distinguish one from another. The "ordinary form" here used means the individuals that well coincide with descriptions and figures for respective species given by Ohwi (1975) and Makino (1961). However, there are a considerable number of variants which have intermediate morphological characters and are more difficult to identify. Ohwi (1941) noted that hybridization of various species combinations would be related to the occurrence of such variants.

Since Ohwi's (1941) publication, no noticeable taxonomic study of this group has been carried out except for the work of Tateoka (1987), who clarified the taxonomic status of *Poa crassinervis*. Boundaries between *P. nipponica*, *P. acroleuca* and *P. hisauchii*, as well as the extents of hybridization among them, still remain obscure and should be the subject of futher taxonomic enquiry (cf. Tateoka 1985). Studies should also be made of whether *P. nipponica* is an independent taxon, because it was sometimes stated that *P. nipponica* itself might be a hybrid between *P. acroleuca* and *P. annua* (Ohwi 1982).

* Institute of Biological Sciences, University of Tsukuba, Tsukuba 305. 筑波大学 生物科学系.

Tateoka (1985) reported chromosome counts for plants of this group and reviewed earlier cytological reports for them, pointing out that plants referred to as *Poa nipponica* were hexaploid (2n=42) while the other four species were tetraploid (2n=28). Tateoka (1985) also noted that nuclear plates of *P. nipponica*, *P. annua* and *P. crassinervis* included small chromosomes which were not observed in *P. acroleuca* and *P. hisauchii*. No detailed karyotype analysis or observation of meiotic chromosomes of these five species in Japan have been made. The karyotype of *P. annua* occurring in England was studied in detail by Koshy (1968).

The observations that *Poa nipponica* and related species are composed of tetraploids and hexaploids and that there would be interspecific differences in karyotype (Tateoka, 1985) are suggestive of the usefulness of chromosome features in dissolving the taxonomic ambiguity indicated above. In order to solidify the base of the study along this line, I have attempted to answer the following three questions using ordinay forms of *P. nipponica*, *P. acroleuca*, *P. hisauchii* and *P. annua* which grow in Tsukuba City and its vicinities in Ibaraki Pref. (1) Does *P. nipponica* exist as an independent taxon? (2) Are there clear differences in karyotype among ordinary forms of the four species? (3) If some interspecific karyotypical differences exist, how significant are the differences taxonomically?

Material and methods Plants were collected at several localities, shown in Tab. 1.

Mitotic chromosomes were observed in root tip meristematic cells. Plants were grown in a water culture regime to obtain actively growing root tips. The elongated roots were cut at a point 2 cm from the tip (pieces 2 cm long gave the best result). The root tips, together with other parts of the root pieces, were pretreated for 2 hr at 20°C in 0.02% colchicine and fixed in a mixture of ethanol 3: glacial acetic acid 1 at 5°C for 1 hr. After being kept in the same 3:1 solution at room temperature for 3 weeks, the root tips were stained with 1% aceto-orcein at 5°C for 2 days, and the solution containing the sample was gently heated to boil over an alcohol flame: the root tips were left in the solution for about 10 min before cutting into slices of about 0.5 mm thick. Slices were squashed on glass microscope slides in 45% acetic acid. Good metaphase plates were photographed. Chromosomes were measured from photographic prints.

Tab. 1. Chromosome numbers of the plants examined.

Species and locality	No. of colonies	No. of individuals	2n	Specimens in reference*
Poa annua L.				
Tsukuba City, Amakubo 20 m alt.	5	5	28	3507, 3510, 3553, 3705, 3710
Poa acroleuca Steud.				
Tsukuba City, Amakubo 20 m alt.	4	7	28	3531, 3716, 3734, 3735
Tsukuba City, Takezono 20 m alt.	1	1	28	3709
Niihari-gun, Niihari-mura 60 m alt.	1	1	28	3719
Poa hisauchii Honda				
Makabe-gun, Makabe-cho, Yubukurotoge 150 m alt.	1	1	28	3633
Do., Jousotoge 200 m alt.	1	1	28	3641
Niihari-gun, Yasato-machi 180 m alt.	1	2	28	3614, 3615
DO., 140 m alt.	1	1	28	3639
Poa nipponica Koidz.				
Nishiibaraki-gun, Iwama-cho, Nagasawa 190 m alt.	1	1	42	3585
Tsukuba City, Amakubo 20 m alt.	3	4	42	3733, 3711, 3620
Tsukuba City, Takezono 20 m alt.	6	9	42	3740, 3706, 3712, 3736, 3713, 3738

* Plants used for chromosome observations often died during water culture, and the voucher specimens could not be prepared. Instead, "specimens in reference" were prepared from the field plant which had a similar appearance and was growing in close proximity to the plant used for water culture. These specimens are deposited in the herbarium of TNS (National Science Museum). The collection numbers are the author's.

For observation of meiosis, anthers taken from florets before the ear opened were stained in 2% aceto-carmine at 5°C for 1 day.

For pollen observations, mature but undehisced anthers were used. The grains were stained for 1 day with lactophenol-cottonblue.

Results Chromosome numbers of the plants collected from the field are shown in Table 1. The number 2n=42 was found in *Poa nipponica*, and 2n=28 in the other three species in agreement with Tateoka's (1985) report.

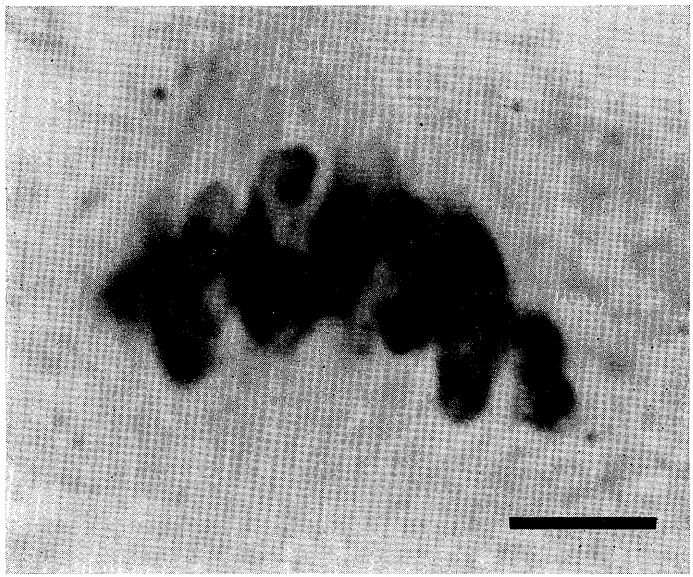

Fig. 1. Meiotic chromosomes at metaphase I in PMC of *Poa nipponica*. Bar indicates 5 μm.

The self-perpetuating nature of *Poa nipponica* was strongly suggested during my field observations. Populations of *P. nipponica* which were developed at sites lacking both *P. acroleuca* and *P. annua* were sometimes encountered, for example in various locations on the sides of Mt. Tsukuba. Plants of *P. nipponica* cultivated in the experimental garden set a lot of seed without exception. Chromosome observations confirmed that the seedlings yielded from the seed had 2n=42 like the parental plants. Observations of meiotic divisions of *P. nipponica* showed that it has regular meiosis. None of univalents, chromosome bridges or lagging chromosomes were seen (Fig. 1). High pollen stainability was also found in *P. nipponica*.

Somatic nuclear plates of the four species examined are shown in Figs. 2, and the chromosomes are serially arranged in Fig. 3. In *Poa annua* (Fig. 2-A), two pairs of chromosomes were distinctly longer than the rest, and a secondary constriction was occasionally found in chromosomes of the third pair. Chromosomes of *P. acroleuca* (Fig. 2-B) and *P. hisauchii* (Fig. 2-C) varied more

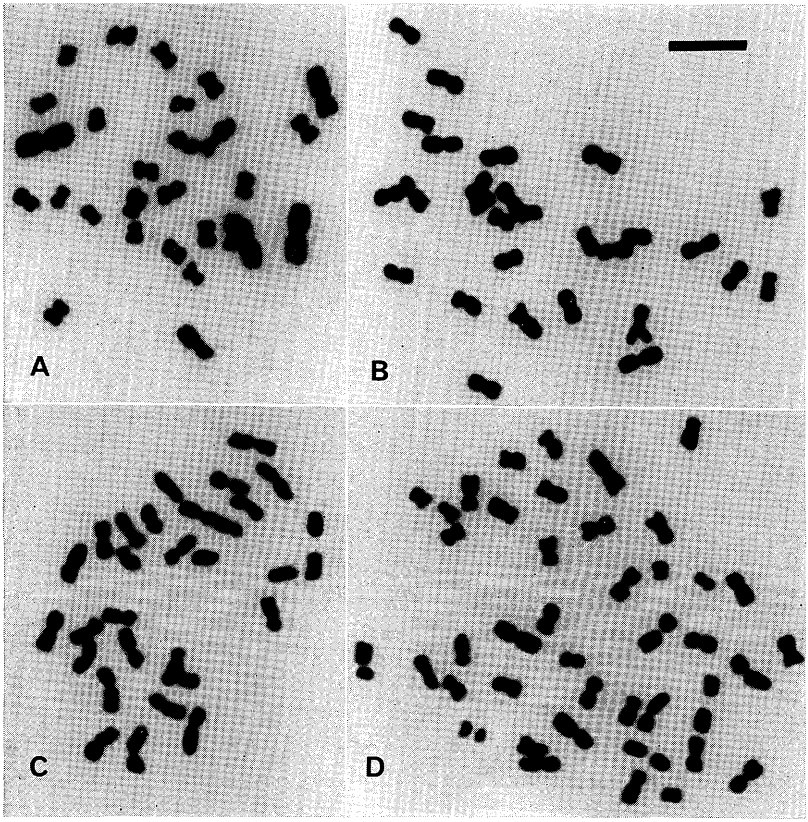

Fig. 2. Somatic chromosomes at metaphase in root tip cells. A. *Poa annua*, B. *P. acroleuca*, C. *P. hisauchii*, D. *P. nipponica*. Bar indicates 5 μm.

gradually in length. In *P. nipponica* (Fig. 2-D), the chromosomes varied considerably in size. All chromosomes of the four species were metacentric or sub-metacentric.

In order to get clear pictures of the karyotype differences between the four species, the following two indices were calculated for chromosomes of every metaphase plate examined: length ratio of the two longest chromosomes to the two shortest chromosomes (abbreviated to LR), and a coefficient of variability as regards the length of a set of chromosomes (abbreviated to CV). Both LR and CV were shmewhat variable according to metaphase plates even among

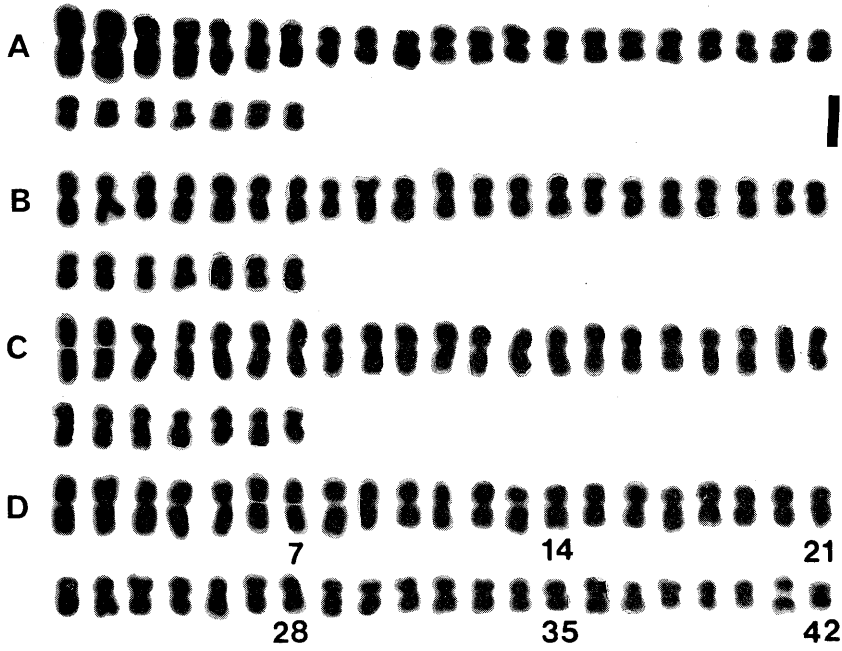

Fig. 3. Serial arrangement of the metaphase chromosomes in root tip cells. A. *Poa annua*, B. *P. acroleuca*, C. *P. hisauchii*, D. *P. nipponica*. Bar indicates 3 μm.

those of the same individual (this variation seemed to have been caused by some artificial factors related to the measurements), and the LRs and CVs obtained in each species were expressed as their ranges, standard errors and means. The figures obtained are indicated in Tab. 2. It may be clear from this table that the four species can be grouped as follows: (1) *Poa annua* which has the largest values of LR and CV, (2) *P. acroleuca* and *P. hisauchii* with the smallest LRs and CVs, (3) *P. nipponica* which is intermediate between the above two classes in LR and CV. Differences in LR and CV between these three groups were statistically significant ($P<0.001$, randomization test), while the differences between *P. acroleuca* and *P. hisauchii* were not significant ($P>0.2$ for LR, $P>0.9$ for CV).

Discussion As described above, the ordinary form of *Poa nipponica* had regular meiosis, high pollen stainability, good seed set and selfreproductivity. These observations made in laboratory were in good accord with the assumptions

Tab. 2. Values of the two karyotypical indices* in the four species of *Poa*.

Species	No. of individuals	No. of metaphse plates	LR			CV		
			Range	Mean	S.E.	Range	Mean	S.E.
Poa annua L.	5	9	3.0–3.7	3.3	.083	.35–.45	.39	.0093
Poa acroleuca Steud.	9	20	1.6–2.1	1.8	.024	.14–.19	.17	.0027
Poa hisauchii Honda	5	17	1.7–2.0	1.8	.029	.14–.20	.17	.0041
Poa nipponica Koidz.	14	22	2.2–2.9	2.5	.036	.20–.27	.24	.0034

* LR: Length ratio of the two longest chromosomes to the two shortest chromosomes.
CV: Coefficient of variability of the length of a set of chromosomes.

obtained from field observations of the populations of this form. It must be admitted, therefore, that the ordinary form of *P. nipponica* exists as a real taxon which perpetuates itself and is not an ephemeral being like an F_1 plant. In other words, *P. nipponica* itself is a good species and is not a hybrid, although it seems highly probable that some interspecific hybridization coupled with polyploidization has given rise to *P. nipponica*.

The karyotype analysis showed that plants of the four species examind are separated into three groups, two of which are tetraploid and one hexaploid. The results have the following taxonomic implications. First, since *Poa acroleuca* and *P. hisauchii* are quite similar in haryotype, chromosome features can not be expected to be helpful in clarifying the taxonomic ambiguity related to the boundary between these two species. Secondly, the results on the karyotype of *P. annua* here obtained on the basis of the Japanese material have coincided for the most part with those reparted from England by Koshy (1968), and the evident differences in karyotype between this species and the other two tetraploids, *P. acroleuca* and *P. hisauchii*, strongly suggest that karyotypical observations may be useful in cases where the hybridization between *P. annua* and either of the other two tetraploids should be subjected to analysis.

Finally, it has been proved that chromosome features must be helpful in delimiting *Poa nipponica*. The F_1 hybrids between hexaploid *P. nipponica* and the related tetraploid species must be pentaploids with $2n=35$ and can easily be recognized as such by chromosome observations. Further, unless these species are unstable in chromosome size, it is possible by karyotype observations to

distinguish hexaploid *P. nipponica* from the 2n=42 plants which can be produced from the union of reduced and unreduced gametes of the tetraploid species. Table 3 shows six possible combinations of the tetraploid plants which can give rise to the 2n=42 plants of the latter nature, together with LR and CV values expected in respective combinations. When *P. annua* participates in the production of the 2n=24 plants, LRs of the resultant plants ($3.3+\alpha$) must be larger than the LR of *P. nipponica* (2.5). In the case of 2n=42 plants raised from the union of reduced and unreduced gametes of *P. acroleuca* and/or *P. hisauchii*,

Tab. 3. Estimated values of the two karyotypical indices* for the supposed 2n=42 plants which are assumed to have been derived from the union of reduced and unreduced gametes.

Combination of parental tetraploid species giving a reduced and an unreduced gamete	LR	CV
Poa annua×*Poa annua*	$3.3+\alpha$**	0.39
Poa annua×*Poa acroleuca*	$3.3+\alpha$	0.17–0.39
Poa annua×*Poa hisauchii*	$3.3+\alpha$	0.17–0.39
Poa acroleuca×*Poa acroleuca*	$1.8+\alpha$	0.17
Poa acroleuca×*Poa hisauchii*	$1.8+\alpha$	0.17
Poa hisauchii×*Poa hisauchii*	$1.8+\alpha$	0.17

* LR: Length ratio of the two longest chromosomes to the two shortest chromosomes. CV: Coefficient of variability of the length of a set of chromosomes.

** "$+\alpha$" is necessary to LRs of the supposed 2n=42 plants because of the following fact. Measurements of chromosomes sizes are accompanied by observational errors which occur in a certain range. The chromosome recognized to be the longest is the one which is accompanied by the largest positive error among the chromosomes belonging to the largest group. As the number of chromosomes belonging to the largest group increase along with polyploidization, the possibility that a large positive error deviated from the standard attaches to such chromosomes increases concomitantly. Consequently, the length of the chromosome to be recognized as the longest tends to hold a larger value in the case of 2n=28+14 than in the case of 2n=28. Similarly, the length of the chromosome recognized as the shortest tends to show a smaller value in the 2n=42 plants. Thus, the LR as defined above may automatically become somewhat larger in the supposed 2n=42 plants than in the original 2n=28 plants.

the expected CVs (0.17) must be smaller than the CV of *P. nipponica* (0.24).

As stated earlier, there are many plants with morphological features intermediate between ordinary forms of *Poa nipponica* and related species. The results of the present work show that the clarification of taxonomic status of such intermediate plants will be facilitated by the observation of their chromosome features.

I express my thanks to Professor Mitsuo Chihara of the Institute of Biological Science, the University of Tsukuba, and Dr. Tuguo Tateoka of Department of Botany, the National Science Museum, who have continuously guided me during the couse of this work and read the manuscript carefully for the improvement thereof. I am indebted to the members of the Laboratory of Biology, Kanagawa University, who gave useful advice on the techniques for chromosome observations. I also thank to Drs. Terumitsu Hori, Yoshiaki Hara, Isao Inouye and Yuichi Kadota for their generous advice and encouragement throughout the work.

References

Koshy, T.K. 1968. Evolutionary origin of *Poa annua* L. in the light of karyotypical studies. Can. J. Genet. Cytol. **10**: 112–118. Koyama, T. 1964. Gramineae. *In* S. Kitamura *et al.*, Colored illustrations of herbaceous plants of Japan III: 301–388. Hoikusha, Osaka. Makino, T. 1961. New illustrated flora of Japan. Hokuryukan, Tokyo. Ohwi, J. 1941. Gramina Japonica 1. Acta Phytotax. Geobot. **10**: 94–135. ——— 1975. Flora of Japan, new ed., revised and enarged. Shibundo, Tokyo. ——— 1982. Poaceae. *In* Satake, Y. *et al.*, Wild flowers of Japan; Herbaceous plants I: 85–126. Heibonsha, Tokyo. Tateoka, T. 1985. Chromosome numbers and taxonomic implications in the genus *Poa* of Japan. Bot. Mag. Tokyo **98**: 413–437. ——— 1987. Identity and distribution of *Poa crassinervis* (Poaceae). Acta Phytotax. Geobot. **38**: 176–186.

* * * *

オオイチゴツナギは一般に独立種として扱われているが，外部形態の特徴から，あるいはスズメノカタビラとミゾイチゴツナギの雑種ではないかといわれてきた植物である（大井 1982）。近年の館岡（1985）の染色体数の調査によって，オオイチゴツナギは 2n=42 の 6 倍体植物で，ともに 2n=28 の 4 倍体であるスズメノカタビラとミゾイチゴツナギの単純な雑種ではないことが示された。オオイチゴツナギの種としての独自性

をさらに検討するために，関連植物の典型的な型を用い，栽培による自己増殖性の検討，国内各地においての生育地の調査，および減数分裂と核型の観察をおこなった。

その結果，1) オオイチゴツナギは正常な減数分裂をおこない，高い自己増殖性をもつこと，2) 核型において，①スズメノカタビラ，②ミゾイチゴツナギとヤマミゾイチゴツナギ，③オオイチゴツナギ，の3群は，それぞれ特異性をもつこと，が明らかになった。これらの結果は，オオイチゴツナギが独立種であることを示している。また，本研究で明らかにした各種の核型の特異性は，実際に各地でみつかっているオオイチゴツナギとミゾイチゴツナギ，およびオオイチゴツナギとヤマミゾイチゴツナギのいわば中間的な形態的特徴をもつ植物の分類学的検討に利用し得るものである。

□Martin, B. (ed.): **Plant vacuoles** 562 pp. 1987. Plenum Press, New York. $125. 1986年フランスのソフィアで行われた液胞に関するシンポジウムのまとめである。液胞はかつては老廃物の捨て場とか，膨圧によって植物体を保持するという程度の役割しか認識されていなかった。しかし最近では液胞がその膜面を介して，光合成をはじめ植物の生理活動に多くの重要な役割を果たしていることがわかってきており，このようなシンポジウムが行われるようになった。本書には68件の発表が記録されている。各報告は非常に細かい生理作用についてのものばかりで，直接に分類学に関与するものは見当たらないが，旧い認識を改めるのに役立つ。それよりも序文の中で「菌類と植物」という表現がなされていて，生物界のこういう分け方が進みつつあることを感ずる。 (金井弘夫)

□東京都目黒区（編）：**街の自然12か月 資料編 目黒区産動植物目録** 80 pp. 1985. 東京都目黒区役所．新刊としてはやや遅いが，訂正すべき箇所があり，またサンケイ児童出版文化賞を受賞した資料集でもあるので，あえて紹介したい。本書は，先に発行された「街の自然12か月 めぐろの動植物ガイド」の資料集で主にシダ植物，種子植物，昆虫，鳥類の目録を収めている。目黒区は面積約 14 km^2，緑地率約 11% で，植物目録の種数は植栽種も含めて716種，その内，自生種と帰化種のみでは401種，帰化率は約26%である。カンアオイ属は前川文夫が同定し，ヒメカンアオイ *Asarum takaoi* F. Maekawa のみが「稀 野生化」として記録されている。しかし，その種名をコバノカンアオイ *Heterotropa variegata* (A. Braun et Bouché) F. Maekawa に訂正する。前川文夫が現地調査した時点で，その個体は葉が円腎形，凹頭，長柄という本種の特徴を有していたが，1個体のみのため採集しなかった。その同定結果は私が手紙で伝えたが，何らかの手違いにより，この記録ミスが生じたようである。なお，その後その個体は標本に作成され，目黒区役所が保管している。 (前川由己)